Folate and Prostate Cancer:
A Case-Control Study

By

Rachel Linz

A THESIS

Presented to the Department of Public Health and Preventive medicine
and the Oregon Health and Science University
School of Medicine
in partial fulfillment of
the requirements for the degree of
Master of Public Health

May 2009

Department of Public Health and Preventive Medicine

School of Medicine

Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Master's thesis of

Rachel Linz

has been approved

Jackilen Shannon, PhD

Mark Garzotto, MD

Dawn Peters, PhD

Table of Contents

List of Tables and Figures

Acknowledgments

I would first like to thank my committee members, Dr. Jackie Shannon for inviting me to conduct the blood folic analysis for my internship and for making this project possible, Dr. Mark Garzotto for being a key member of the study team and for providing valuable clinical expertise, and Dr. Dawn Peters for sharing her extensive statistical knowledge and for keeping me on track.

I would also like to thank the OHSU Department of Public Health and Preventive Medicine, especially Dr. Katie Riley and Tree Triano, for all of their help over the past two years.

Finally, I would like to thank my family, friends, and classmates for all of their support.

Abstract

Background: Prostate cancer is the most prevalent cancer in men in the United States. It is estimated that in the US 186,000 men will be diagnosed with prostate cancer in 2008 and that the lifetime probability of being diagnosed with prostate cancer is 16.7%. Of the currently known risk factors for prostate cancer, such increasing age, race and family history, few are modifiable. Due to geographic variation in prostate cancer incidence, researchers have been investigating environmental factors, such as diet, that may play a role in prostate cancer risk. Folate is one such dietary factor that has been thoroughly investigated with regard to colorectal cancer due to its role in one-carbon metabolic pathways. However, studies investigating the role of folate in prostate cancer risk have reported conflicting results.

Methods: We conducted a case-control study of men with biopsy confirmed prostate cancer, men with negative biopsies, and a third group of clinic controls with no history of prostate abnormalities. Study subjects completed an interviewer administered, validated, diet history questionnaire prior to biopsy (or primary care appointment), as well as providing demographic and risk factor information and a pre-biopsy blood specimen. Blood specimens were analyzed for folic acid content using an automated chemiluminescence assay, and the association between folic acid concentration and reported intake of dietary and synthetic folate and folic acid was examined. In addition, multiple logistic regression models were built to examine the significance of folic acid concentration on prostate cancer risk when combined with known risk factors.

Results: Blood folic acid concentration was not significantly associated with prostate cancer. Correlations between blood folic acid concentration and reported dietary intake of folate or folic acid were low.

Conclusion: Although others have reported a significant association between folate and prostate cancer, we did not observe such an association. The timing of exposure to folate may affect prostate cancer risk. Further longitudinal studies examining folate intake are recommended.

Background & Significance

Prostate cancer (PCa) is the most prevalent cancer in men in the United States. It is estimated that in the US 186,000 men will be diagnosed with prostate cancer in 2008 and that the lifetime probability of being diagnosed with prostate cancer is 16.7% [11]. Of the currently known risk factors for prostate cancer, such as age, race and family history, few are modifiable.

Incidence of invasive prostate cancer is highest in Europe, North America and Australia, whereas the lowest incidence is found in Japan and other Asian countries [2]. Results from migration studies have demonstrated that the risk of developing prostate cancer increases when men move from a low-risk country to a high-risk country, suggesting that environmental factors such as lifestyle and dietary habits are associated with the risk of developing invasive prostate cancer [3]. Thus, recent epidemiologic studies have focused on assessing potential modifiable risk factors for prostate cancer, such as diet.

One dietary component that has been implicated in the pathogenesis of several cancers is folate. Folate is an essential B vitamin found in leafy green vegetables, whole grains, and some fruits. Folate in this form is known as 5-methyltetrahydrofolate (5-MTHF) or pteroylpolyglutamate. Starting in 1998, the United States Food and Drug Administration mandated that enriched flours and cereals be fortified with folic acid (140µg/100gm serving), the fully oxidized synthetic form of folate, in order to reduce the incidence of neural tube defects (NTDs) [4]. Folic acid, or pteroylmonoglutamate, is also the form of folate found in supplements. Folic acid fortification has resulted in an

increased intake among adults in the United States [5]. Throughout this paper "folate" will refer to 5-MTHF and "folic acid" will refer to pteroylmonoglutamate.

Folate is an essential factor in the one-carbon metabolic pathway that is involved in DNA methylation, synthesis, and repair. Insufficient levels of folate can compromise nucleotide synthesis and lead to misincorporation of uracil instead of thymidine in DNA, causing DNA instability and increased mutations and chromosomal breaks [6]. In several studies of colorectal cancer, the most frequently studied cancer in relation to one-carbon metabolism, high folate status has been reported to be associated with a modestly reduced risk for cancer development in prospective studies [7]. However, the first randomized controlled trial investigating the potential antineoplastic effect of supplementary folic acid in subjects with a history of colorectal adenomas found an increased risk of advanced lesions in the supplementation group compared to the placebo group [8]. This unexpected result suggested that folic acid plays a dual role in the neoplastic process depending on the timing of exposure.

In addition to its role in nucleotide synthesis, folate is directly involved in the formation of *S*-adenosylmethionine (SAM), which provides methyl groups for DNA methylation, a common method of gene regulation. DNA methylation involves the covalent bonding of a methyl group to a cytosine that precedes a guanosine in the DNA sequence (a CpG dinucleotide). CpG dinucleotides are uncommon in the genome as a whole, but are often clustered in small stretches of DNA called "CpG islands." These islands are often found in the promoter regions of genes. About 80% of the CpG dinucleotides that are not associated with CpG islands are methylated, whereas CpG

islands, especially those found in promoter regions, are usually unmethylated. Methylation of CpG islands in promoter regions reduces or silences gene expression [9].

Hypermethylation of certain genes has been observed in cancer cells and tissues for more than 20 years. Recent improvements in detection of methylation through methylation-specific PCR (MSPCR) have increased the body of knowledge on the association between gene methylation status and cancer [10]. Hypermethylation has been reported to occur in genes responsible for cell cycle regulation (RASSF1a, p16/CDKN2a), DNA repair (GSTP1, MGMT), and tumor growth and progression (APC, RARB2) and has been seen in several types of cancer [11-13]. In prostate cancer patients, promoter hypermethylation of certain genes has been shown to provide significant value in diagnosis and prognosis [14,15].

Due to the multiple roles of folate in one-carbon metabolic pathways, it is not surprising that studies examining the association between folate and prostate cancer have reported conflicting results. Although some studies have suggested that high folate status may increase risk of prostate cancer [16,17], these associations were not statistically significant. Others have reported a protective effect of folate [18-20] and still others have reported no association at all [21,22]. The Aspirin/Folate Polyp Prevention Study [23], the first randomized controlled trial of folic acid supplementation that assessed prostate cancer as an endpoint, found that although baseline dietary intake of folate was inversely associated with prostate cancer, folic acid supplementation was associated with an increased risk of prostate cancer. These intriguing results support the need for further

study to elucidate the relationship between folate intake and prostate cancer. A summary of these studies is provided in Table 1.

There are several potential reasons for the discrepancy in reported findings. First, the questionnaires used assess folate intake in different ways and thus provide different estimates of folate intake. Second, those studies using serum and blood samples used a variety of methods that cannot be reliably compared [24]. There is no currently accepted "gold standard" for folate analysis.

Table 1. Summary of studies examining the association between folate and PCa

First Author and Year	Location	Study Design	Method of Folate Analysis	Results
Vlajinac 1997	Serbia	Case-control	Diet questionnaire	Null
Weinstein 2003	Finland	Case-cohort	Diet questionnaire; serum sample	Null
Hultdin 2005	Sweden	Nested case-control	Serum sample	Positive (not significant after adjustment for B12)
Pelucchi 2005	Italy	Case-control	Diet questionnaire	Negative
Rossi 2006	Australia	Prospective cohort	Serum sample; blood sample	Negative
Stevens 2006	Europe	Prospective cohort	Diet questionnaire	Negative (not significant)
Johansson 2008	United States	Nested case-control	Blood sample	Positive (not significant)
Figueiredo 2009	United States	Randomized Controlled Trial	Serum sample; Diet questionnaire	Negative for dietary folate; positive for folate supplementation

Significance: Prostate cancer is a source of significant health burden in men in the United States. Identification of modifiable risk factors is essential to reducing the incidence of this disease. Due to increased folic acid intake since the mandatory fortification with folic acid of cereals and flours in 1998, it is critical to determine whether this increased folic acid intake is in fact advisable to all members of the US

population. In contrast to previous studies, we will obtain information from three groups: men with biopsy-confirmed prostate cancer, men with negative biopsies, and men with a normal PSA and no history of prostate conditions. The goals of this study are to determine whether circulating blood folic acid concentration is associated with measures of reported dietary intake of folate, and to determine whether blood folic acid concentration is associated with prostate cancer after accounting for known risk factors.

Specific Aims

- Primary Aim #1: To evaluate the association between blood folic acid concentration and dietary folate consumption as measured by a food frequency questionnaire.
    - Hypothesis for Primary Aim #2: Blood folic acid concentration will be significantly correlated with dietary folate consumption.
- Primary Aim #2: To evaluate the association between blood folic acid concentration and prostate cancer.
    - Hypothesis for Primary Aim #1: Those with high blood folic acid concentration and those with low blood folic acid concentration will have differing odds of having prostate cancer

METHODS

Overview

This case-control study added blood folic acid data to existing data collected for a Diet and Prostate Cancer (DPC) study conducted between February 2002 and June 2006. The goal of the DPC study was to examine dietary influences on prostate cancer in combination with known risk factors [25]. The goal of this study was to determine whether blood folic acid concentration is associated with prostate cancer risk and to what extent blood folic acid concentration correlates with reported dietary intake. Finally, we built a multivariate model to assess the relationship between prostate cancer and the existing dietary and risk factor data in combination with blood folic acid data.

Recruitment and Informed Consent

Identification and selection of subjects has been described previously [25]. Briefly, cases and biopsy-negative controls included men with an elevated prostate-specific antigen (PSA) level who were referred by their primary care physician to the VA urologic oncology (UO) clinic for a prostate biopsy. Clinic controls were identified as those men >50 years of age who had an upcoming appointment at the primary care clinic at the VA and who had a normal PSA reading within the previous year, no history of prostate conditions, and were not currently being treated for prostate conditions, dementia, or existing cancer.

Research coordinators contacted each man to determine whether he would be willing to participate. Participants were asked to come to the VA prior to scheduled biopsy or primary care appointment to complete the Informed Consent process and to

provide demographic and risk factor information, complete the Diet History Questionnaire, and provide a blood sample.

For this analysis, men who were diagnosed with prostatic intraepithelial neoplasia (PIN) as a result of their biopsy were excluded due to the known status of PIN as a precurser to prostate cancer (n = 36). Men who did not provide a blood sample for folic acid analysis (n = 124) and men who did not complete the dietary and risk factor questionnaires (n = 11) were also excluded, leaving a total of 537 subjects (120 cases, 236 biopsy negatives, and 181 PSA normal controls) for this analysis. Details of those subjects who were identified and those who were included in this analysis are shown in Figure 1. All participants provided written informed consent according to both the PVAMC and OHSU Institutional Review Boards' requirements and approval.

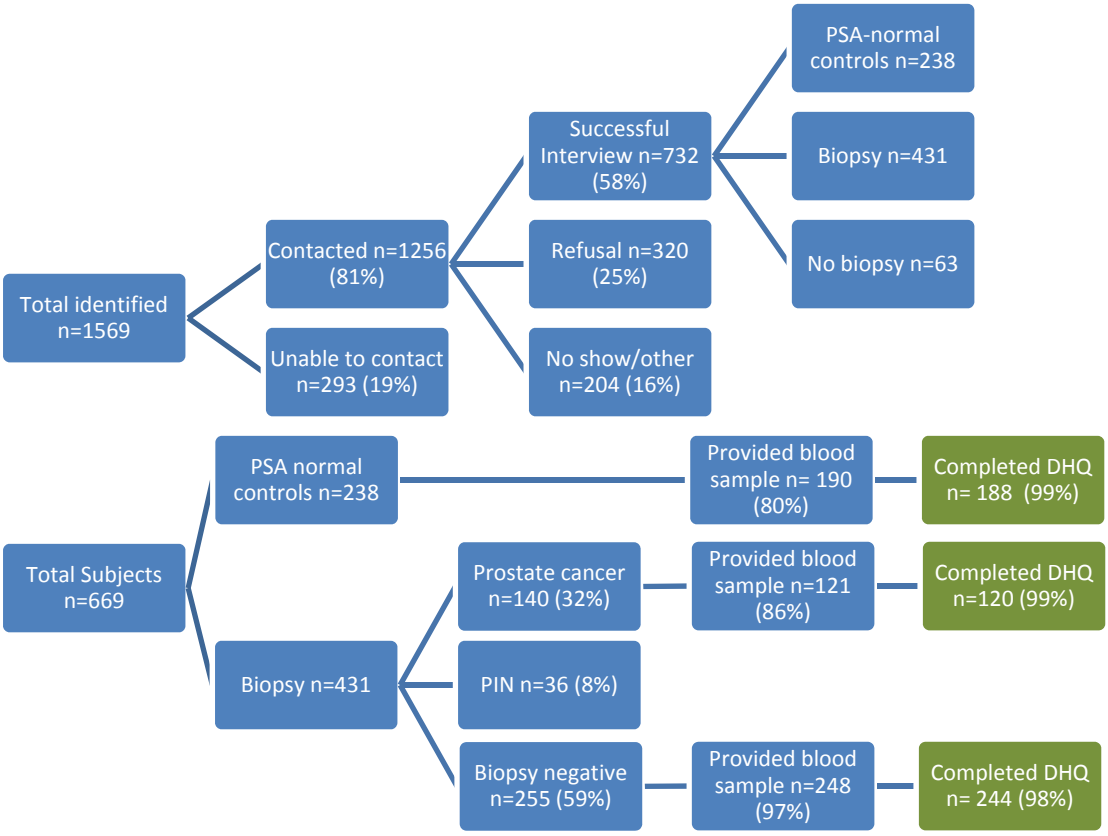

Figure 1. Sample population

Measurement of Predictor Variables

Diet and Other Risk Factors

Information on diet history was obtained using an adapted version of the National Cancer Institute Diet History Questionnaire (DHQ)[26]. Participants were asked to recall their usual dietary intake of 124 foods, nutrient supplement use, beverage consumption and use of herbal remedies for the previous 12 months. Intake of individual nutrients was determined by analysis of the DHQ through the NCI Diet*Calc

software. Intake of folate and folic acid was calculated in several ways. First, natural folate from food sources was captured as Food Folate. Second, synthetic folate from fortified foods was captured as Synthetic Folate. Supplemental Folic Acid was calculated as folic acid coming from vitamin supplements. Finally, because food folate has lower bioavailability than synthetic folate [27], a Dietary Folate Equivalents variable was calculated as the sum of Food Folate and (Synthetic Folate multiplied by 1.7). The risk factor questionnaire requested information on age, race, ethnic origin, body mass index (BMI), education, family history of cancer, use of non-steroidal anti-inflammatory drugs (NSAIDs), supplement use, comorbid conditions, occupational history, alcohol consumption and smoking.

Blood Folic acid Analysis

A blood specimen was drawn from each participant using a vacutainer tube. Tubes were stored at 5-10°C in the dark no longer than 5 hours before processing. 0.1mL of whole blood was aliquoted to each of two cryovials and 0.5mL freshly prepared 0.8% sodium ascorbate was added to hemolyze red blood cells and stabilize folic acid. Tubes were inverted gently to mix and allowed to stand at room temperature (22-27°C) for complete hemolysis. Cryovials were then frozen at -80°C until analysis. All samples were prepared and analyzed at the General Clinical Research Center at OHSU.

Samples were analyzed in batches containing similar numbers of cases and each of the two control groups by laboratory personnel blinded to the case-control status of the specimens. Folic acid concentrations were determined using a competitive, ligand-labeled, protein-binding chemiluminescent assay on an IMMULITE 1000 (Siemens

Healthcare Diagnostics, Deerfield, IL). Control samples (Lyphochek Whole Blood Control, Bio-Rad Laboratories, Hercules, CA) were run between every 60 samples. Intraassay and interassay CVs were 6.6% and 12.1%, respectively.

Measurement of Outcome Variable

Presence of prostate cancer was determined via histological examination of biopsy tissue. Severity of disease was characterized by TNM staging and Gleason score.

Statistical Analysis

The objectives of the current study were to (1) determine the distribution of whole blood folic acid concentration in our sample, (2) assess the homogeneity of the distribution of folic acid concentration in cases and controls, (3) evaluate the association between blood folic acid concentration and reported dietary intake of folate and folic acid, and (4) build a multivariate model using laboratory, dietary and risk factor data to predict the outcome of prostate cancer. All statistical analyses were performed using the Statistical Analysis System (SAS) program, version 9.1 (SAS Institute, Inc., Cary, North Carolina).

First, differences in demographic and risk factor information were evaluated between subjects who did and did not provide a blood sample to assess the presence of bias. Second, correlations were assessed between blood folic acid concentration and all measures of reported dietary intake including food folate, synthetic folate, supplementary folic acid, and dietary folate equivalents. Intake of the different measures of folate and circulating concentration of folic acid were categorized into quartiles based on the distribution in the entire sample for logistic regression analysis.

Dietary and other risk factor and demographic information were used to build multivariate logistic regression models comparing prostate cancer cases with either biopsy negative subjects or clinic control subjects. First, univariate analyses were conducted on the association of prostate cancer outcome with known and possible risk factors (age, race, family history, BMI, smoking), blood folic acid concentration, and dietary factors (alcohol consumption, food folate, synthetic folate, supplementary folic acid, dietary folate equivalents, and vitamin B6 intake). Those variables found to have a significant ($p \leq 0.25$) association with prostate cancer were retained in the initial multivariate model. Variables that were not significant in the initial multivariate model were excluded individually to determine whether their absence significantly (>10%) changed the beta estimates of remaining predictor variables. Had any excluded variable exhibited such a change, it would be retained in the model.

To evaluate the effect of these potential risk factors on prostate cancer of different serverity, cases were grouped according to Gleason score (< 7 and ≥ 7). The methods described above were used to build multivariate logistic regression models comparing all of the PSA normal clinic controls with these two groups of cases.

Due to the fixed sample size of this study, our power to detect significant differences in prostate cancer risk is limited. This study was originally designed to detect differences in fatty acid consumption and the sample size was calculated for this purpose. This study has power of 80% (alpha = 0.05) to detect relative risks for prostate cancer as close to unity as 0.40 and 2.46 between cases and biopsy negatives, and as

close to unity as 0.43 and 2.31 between cases and clinic controls in the highest and lowest quartiles of blood folic acid concentration.

RESULTS

Demographics

Because a number of subjects did not provide blood samples, potential demographic differences between those who did and did not provide blood samples were evaluated and are shown in Table 2. No significant differences were found in age, case/control status, race, BMI, smoking status, family history of prostate cancer, or education.

Demographic characteristics of cases and controls who provided both blood samples and dietary data, listed by subject group, are shown in Table 3. Mean ages for all three groups were similar, but the biopsy negative group had a significantly greater proportion of those under 55 years of age than the cases and clinic controls. All three subject groups were predominantly White, reflecting the racial makeup of Portland-area residents, although the biopsy negative group had a greater proportion of both Asian and Native American/Alaska Native subjects than the other subject groups. Clinic controls were significantly heavier than cases, with a greater proportion of subjects with BMI >35. Clinic controls were also less likely to have a family history of prostate cancer. Biopsy negative subjects were more likely to have never smoked and also had more education than cases.

Folate

Next, the distribution of blood folic acid concentration was examined. The overall mean concentration was 839 ng/mL, and the distribution was strongly right-skewed with a median concentration of 786 ng/mL and a range of 99 ng/mL to 3648

ng/mL. This distribution did not vary significantly by subject group. Among cases, the mean folic acid concentration was 824 ng/mL, the median was 792 ng/mL, and the range was 171-1932 ng/mL. Among biopsy negative controls, the mean folic acid concentration was 846 ng/mL, the median was 783 ng/mL, and the range was 99-3648 ng/mL. Among clinic controls, the mean folic acid concentration was 847 ng/mL, the median was 797 ng/mL, and the range was 126-2088 ng/mL. Histograms showing the distribution of blood folic acid concentration among cases, biopsy negative controls, and clinic controls can be found in Figures 2, 3, and 4, respectively. These figures indicate that the distribution of blood folic acid concentration is fairly similar across subject groups, although the biopsy negative controls had more outlying observations than the other groups.

Next I assessed the distribution of all measures of folate status (blood folic acid concentration, food folate intake, synthetic folate intake, Dietary Folate Equivalents, and use of supplemental folic acid) among the three subject groups (Table 4). None of the distributions were significantly different between cases and either of the two control groups, although the distribution of Dietary Folate Equivalents approached significance when compared between cases and clinic controls ($X^2_3 = 7.60$, $p = 0.055$). Next I assessed the association between blood folic acid concentration and the different measures of dietary folate intake as captured by the DHQ. Correlation coefficients were low to moderate and ranged from -0.003 for food folate to 0.34 for supplemental folic acid (Table 5).

Table 2. Characteristics of Subjects Who Did and Did Not Provide Blood Samples

Characteristic	Provided Blood Sample (n=544)		Did Not Provide Sample (n=124)		p-value
Age, in Years					
Mean Age	64.3		63.6		**0.30**
Range	46-86		50-81		
	N	**%**	**N**	**%**	
Subject Group					**0.31**
Clinic Control	183	33.6	50	40.3	
Biopsy Negative	240	44.1	52	41.2	
Prostate Cancer	121	22.2	22	17.7	
Ethnic Origin					**0.88**
White	479	90.1	107	93.0	
Black	18	3.4	5	4.4	
Hispanic	9	1.7	1	0.9	
Native American	14	2.7	1	0.9	
Asian	2	0.4	0	0	
Other/Missing	20	3.7	10	8.1	
BMI					**0.82**
Less Than 25	95	17.5	19	15.3	
25-29	189	34.7	41	33.1	
30-34	171	31.4	40	32.3	
≥ 35	89	16.4	24	19.4	
Family History of Prostate Cancer					**0.84**
Yes	56	10.3	12	9.7	
Smoking Status					**0.95**
Never	126	23.7	26	22.8	
Former	290	54.5	64	56.1	
Current	126	23.7	26	22.8	
Missing	12	2.2	10	8.1	
Education					**0.39**
≤ 12 years	167	31.4	29	25	
Some College/Tech.	210	39.5	49	42.2	
≥ College graduate	155	29.1	38	32.8	
Missing/Other	12	2.2	8	6.5	

TABLE 3. Frequencies of Demographic and Risk Factor Characteristics

Characteristic	Prostate cancer cases (n=120)		Biopsy negative controls (n=236)			PSA normal clinic controls (n=181)		
	N	%	N	%	p-value*	N	%	p-value*
Age, in years					**0.0006**			**0.2**
≤55	6	5	26	11		15	8.3	
56-65	55	45.8	118	22		83	45.9	
66-75	46	38.3	88	37.3		74	40.9	
≥75	13	10.8	4	1.7		9	5	
Ethnic Origin					**0.04**			**0.11**
White	107	89.9	207	89.6		165	93.2	
Black	8	6.7	6	2.6		4	2.3	
Hispanic	2	1.7	4	1.7		3	1.7	
Native American	1	0.8	9	3.9		4	2.3	
Asian	0	0	2	0.9		0	0	
Other/Missing	2	1.7	8	3.4		5	2.8	
BMI					**0.93**			**0.04**
≤ 25	23	19.2	42	17.8		27	14.9	
25-29	44	36.7	86	36.4		58	32.0	
30-34	40	33.3	77	32.6		53	29.3	
≥ 35	13	10.8	31	13.1		43	23.8	
Family history of prostate cancer					**0.87**			**0.03**
Yes	15	12.5	31	13.1		10	5.5	
Smoking					**0.01**			**0.48**
Never	17	14.2	64	27.5		35	19.6	
Former	70	58.3	122	52.4		98	54.8	
Current	33	27.5	47	20.2		46	25.7	
Missing	0	0	3	1.3		2	1.1	
Education					**0.003**			**0.41**
≤ 12 years	50	41.7	56	24.0		61	34.1	
Some College/Tech.	40	33.3	103	44.2		67	37.4	
≥ College graduate	30	25.0	74	31.8		51	28.5	
Missing/Other	0	0	3	1.3		2	1.1	

*P-value for chi-square difference between cases and respective control groups

TABLE 4. Distribution of Measures of Folate Status

Characteristic	Prostate cancer cases (n=120)		Biopsy negative controls (n=236)			PSA Normal Controls (n=181)		
	N	**%**	**N**	**%**	**p-value***	**N**	**%**	**p-value***
Blood folic acid concentration					**0.44**			**0.85**
≤ 504 ng/mL	30	25.0	53	22.5		48	26.5	
504 – 783 ng/mL	29	24.2	66	28.0		42	23.2	
783 – 1098 ng/mL	26	21.7	63	26.7		45	28.9	
> 1098 ng/mL	35	29.2	54	22.9		46	25.4	
Food folate intake					**0.56**			**0.48**
≤ 200.09 µg	33	27.5	61	25.9		41	22.7	
200.09 – 266.99 µg	28	23.3	68	28.8		38	21.0	
266.99 – 335.46 µg	33	27.5	52	22.0		49	27.1	
> 335.46 µg	26	21.7	55	23.3		53	29.3	
Synthetic folate intake					**0.72**			**0.46**
≤ 89.23 µg	36	30.0	58	24.6		41	22.7	
89.23 – 130.54 µg	30	25.0	50	25.4		44	24.3	
130.54 – 183.7 µg	27	22.5	61	25.9		45	24.9	
> 183.7 µg	27	22.5	57	24.2		51	28.2	
Dietary Folate Equivalents					**0.61**			**0.055**
≤ 387.87 µg	36	30.0	64	27.1		35	19.3	
387.87 – 494.98 µg	34	28.3	57	24.2		43	23.8	
494.98 – 627.35 µg	25	20.8	53	22.5		56	30.9	
> 627.35 µg	25	20.8	62	26.3		47	26.0	
Use of Supplemental Folic Acid	76	63.3	140	59.3	**0.46**	106	58.6	**0.41**

*P-value for chi-square difference between cases and respective control groups

Figure 2. Distribution of blood folic acid concentration among prostate cancer cases

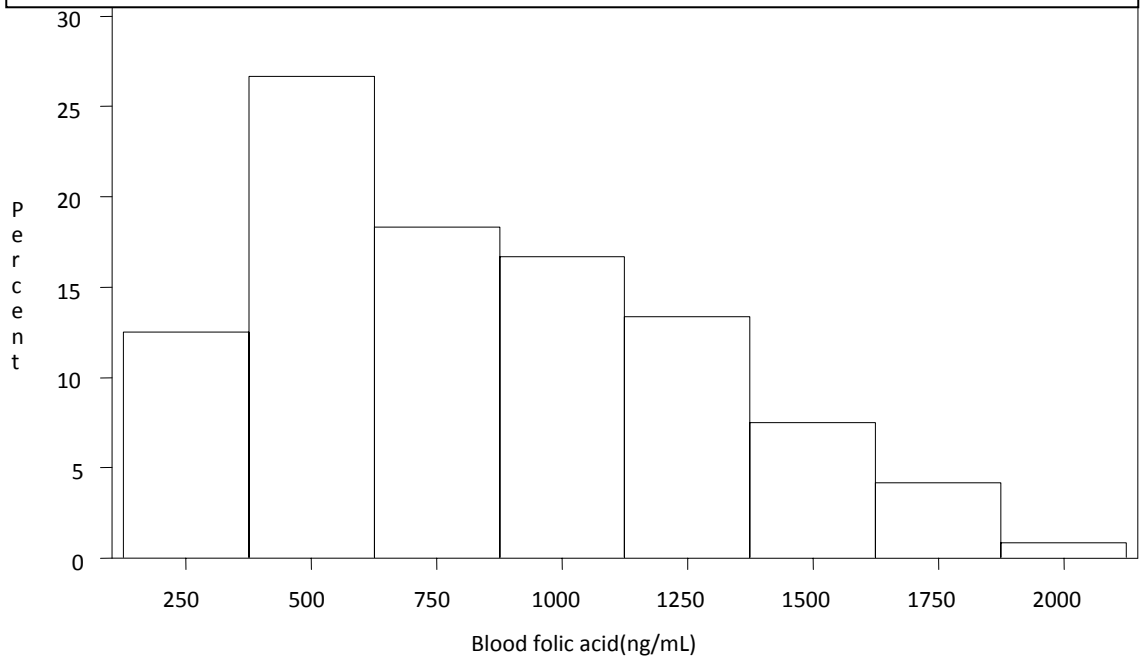

Figure 3. Distribution of blood folic acid concentration among biopsy negative controls

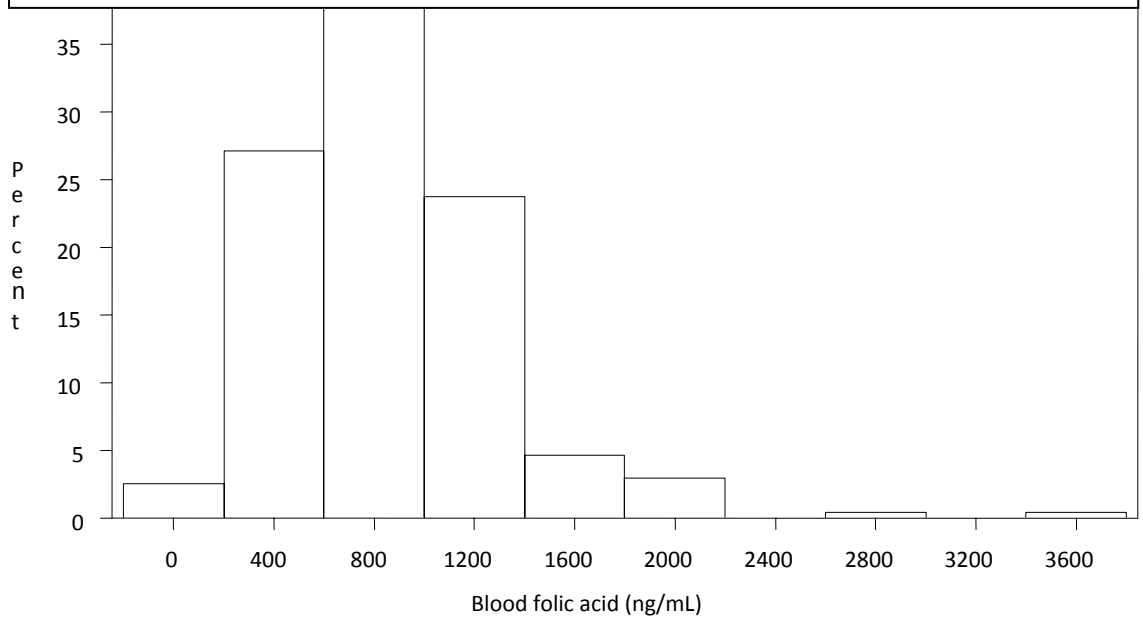

Figure 4. Distribution of blood folic acid concentration among clinic controls

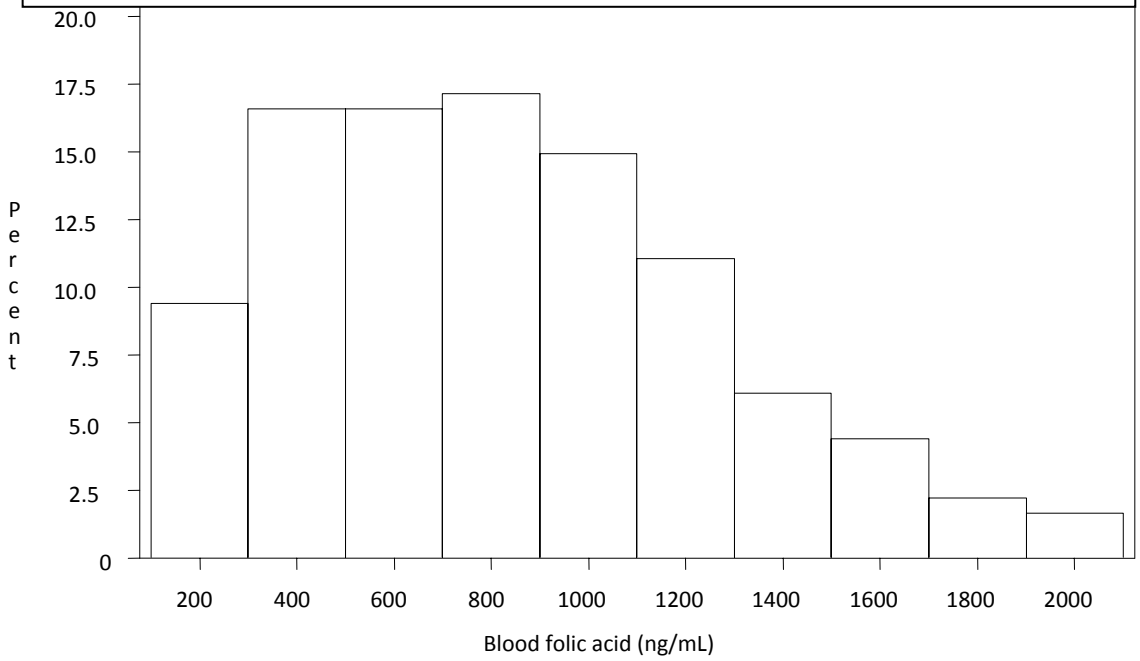

Table 5. Correlation coefficients and corresponding p-values for measures of folate status

	Blood folic acid	Food folate	Synthetic folate	Dietary Folate Equivalents	Supplemental folic acid	Total folate intake
Blood folic acid	1.000	-0.0033 0.938	0.108 0.0123	0.0656 0.129	0.339 <.0001	0.271 <.0001
Food folate	-0.0033 0.938	1.000	0.435 <.0001	0.827 <.0001	0.124 0.0039	0.632 <.0001
Synthetic folate	0.108 0.0123	0.435 <.0001	1.000	0.866 <.0001	0.0861 0.0462	0.632 <.0001
Dietary Folate Equivalents	0.0656 0.129	0.827 <.0001	0.866 <.0001	1.000	0.123 0.0043	0.745 <.0001
Supplemental folic acid	0.339 <.0001	0.129 0.0039	0.0861 0.0462	0.123 0.0043	1.000	0.753 <.0001
Total folate intake	0.271 <.0001	0.632 <.0001	0.632 <.0001	0.745 <.0001	0.753 <.0001	1.000

Logistic Regression Modeling

For logistic regression modeling I ran two separate analyses comparing prostate cancer cases with biopsy negative subjects and prostate cancer cases with clinic controls. I first examined univariate models for each variable of interest, including blood folic acid concentration, dietary folate intake (food folate, synthetic folate and dietary folate equivalents), use of supplemental folic acid, vitamin B6 intake, age, race, family history of prostate cancer, education, alcohol consumption, BMI, smoking, and PSA (for cases compared to biopsy negative subjects only). Models examining categorical variables used design variables to assess the odds of having prostate cancer in each category compared to a reference category. Univariate data for dietary factors are shown in Table 6, and data for other risk factors are shown in Table 7. Those variables found to be significant at the $p < 0.25$ level were included in the initial multivariate model. Variables that were not significant in the initial multivariate model were excluded individually to assess the impact on the remaining predictor variables. Multivariate models were adjusted for Vitamin B6 intake due to its biologic interaction with folate.

Table 6. Univariate comparisons of dietary data between PCa cases and both control groups

	Cases vs. Clinic Controls		Cases vs. Biopsy Negative	
Variable	**Crude OR (95% CI)**	**p-value**	**Crude OR (95% CI)**	**p-value**
Blood folic acid concentration		**0.85**		**0.44**
≤ 504 ng/mL	Referent		Referent	
504 – 783 ng/mL	1.11 (0.57, 2.13)		0.78 (0.42, 1.45)	
783 – 1098 ng/mL	0.92 (0.48, 1.8)		0.73 (0.39, 1.38)	
> 1098 ng/mL	1.22 (0.65, 2.29)		1.15 (0.62, 2.12)	
Dietary folate equivalents		**0.058**		**0.61**
≤ 387.87 µg	Referent		Referent	
387.87 – 494.98 µg	0.77 (0.40, 1.47)		1.06 (0.59, 1.91)	
494.98 – 627.35 µg	0.43 (0.22, 0.84)		0.84 (0.45, 1.57)	
> 627.35 µg	0.52 (0.26, 1.01)		0.72 (0.39, 1.33)	
Food folate		**0.48**		**0.56**
≤ 200.09 µg	Referent		Referent	
200.09 – 266.99 µg	0.92 (0.47, 1.79)		0.76 (0.41, 1.4)	
266.99 – 335.46 µg	0.84 (0.44, 1.58)		1.17 (0.64, 2.16)	
> 335.46 µg	0.61 (0.32, 1.18)		0.87 (0.47, 1.64)	
Synthetic folate		**0.46**		**0.72**
≤ 89.23 µg	Referent		Referent	
89.23 – 130.54 µg	0.69 (0.36, 1.32)		0.71 (0.39, 1.32)	
130.54 – 183.7 µg	0.6 (0.32, 1.15)		0.76 (0.41, 1.42)	
> 183.7 µg	0.78 (0.41, 1.48)		0.82 (0.44, 1.47)	
Use of supplementary folic acid	1.22 (0.76, 1.97)	**0.41**	1.18 (0.75, 1.86)	**0.46**
Vitamin B6 intake (mg)	0.85 (0.63, 1.13)	**0.26**	0.096 (0.75, 1.25)	**0.78**
Alcohol (drinks per day)	1.15 (0.99, 1.34)	**0.063**	1.04 (0.96, 1.14)	**0.35**

Table 7. Additional univariate comparisons between PCa cases and two control groups

	Cases vs. Clinic Controls		Cases vs. Biopsy Negative	
Variable	**Crude OR (95% CI)**	**p-value**	**Crude OR (95% CI)**	**p-value**
Age		**0.22**		**0.003**
≤ 55	Referent		Referent	
56-65	1.66 (0.61, 4.53)		2.02 (0.79, 5.19)	
66-75	1.55 (0.56, 4.29)		2.27 (0.87, 5.9)	
> 75	3.61 (1.01, 12.88)		14.08 (3.37, 58.83)	
Race		**0.13**		**0.046**
White	Referent		Referent	
Black	3.08 (0.91, 10.49)		2.58 (0.87, 7.63)	
Other	0.64 (0.22, 1.88)		0.42 (0.16, 1.14)	
Family history of PCa	2.44 (1.06, 5.64)	**0.036**	0.95 (0.49, 1.83)	**0.89**
PSA		**—**		**0.11**
<4	—		Referent	
4-10	—		1.2 (0.65, 2.2)	
>10	—		2.14 (0.99, 4.64)	
BMI		**0.052**		**0.93**
< 25	Referent		Referent	
25-29	0.89 (0.45, 1.76)		0.93 (0.5, 1.75)	
30-34	0.89 (0.44, 1.77)		0.95 (0.5, 1.79)	
≥35	0.36 (0.15, 0.82)		0.77 (0.34, 1.75)	
Smoking status		**0.49**		**0.016**
Never	Referent		Referent	
Former	1.47 (0.76, 2.83)		2.16 (1.17, 3.98)	
Current	1.48 (0.71, 3.07)		2.64 (1.32, 5.3)	
Education		**0.41**		**0.003**
≤ High School	Referent		Referent	
Some/Technical College	0.73 (0.42, 1.25)		0.44 (0.26, 0.74)	
College Graduate	0.72 (0.4, 1.29)		0.45 (0.26, 0.8)	

Prostate Cancer Cases Compared to Clinic Controls

In the comparison of cases with clinic controls, age, race, BMI, family history of prostate cancer, and three different measures of folate intake (food folate, synthetic folate, and dietary folate equivalents) were found to be significantly associated with prostate cancer at the univariate level, and all were included in the multivariate model. Because dietary folate equivalents comprises both food and synthetic folate, separate

models were built and included either dietary folate or both food and synthetic folate. Blood folic acid concentration was also included in the multivariate model despite its lack of significance at the univariate level. Among the dietary folate variables, only dietary folate equivalents remained significant in the multivariate model, so the model including this variable was pursued. Age, BMI, and family history of prostate cancer remained significant in the multivariate model whereas race and blood folic acid concentration did not. Race and blood folic acid concentration were retained in the model due to the status of race as a known risk factor for prostate cancer and the focus on blood folic acid concentration in this study. Vitamin B6 was also not significant but was retained to provide adjustment related to the folate measures.

Subjects with both the highest and second-highest quartile of intake of dietary folate equivalents were less likely to have prostate cancer, with odds ratios of 0.28 and 0.26, respectively, compared to the lowest quartile of folate consumption (95% CI 0.12-0.64 and 0.084-0.78, respectively). Those with BMI >35 were also less likely to have prostate cancer, with an odds ratio of 0.31 compared to BMI <25 (95% CI 0.13 – 0.78). Having a family history of prostate cancer also increased the likelihood of having prostate cancer with an odds ratio of 2.55 (95% CI 1.04 – 6.24). This model does not exhibit significant lack of fit according to the Hosmer-Lemeshow goodness of fit test (X^2_8 = 10.44, p = 0.23). Details of this model are shown in Table 8.

Table 8. Final multivariate model comparing prostate cancer cases (n= 120) with clinic controls (n=181)

Variable	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Blood folic acid concentration			**0.57**
≤ 504 ng/mL	Referent	Referent	
504 – 783 ng/mL	1.11 (0.57, 2.13)	1.57 (0.77, 3.22)	
783 – 1098 ng/mL	0.92 (0.48, 1.8)	1.16 (0.56, 2.39)	
> 1098 ng/mL	1.22 (0.65, 2.29)	1.46 (0.73, 2.92)	
Dietary folate equivalents			**0.025**
≤ 387.87 µg	Referent	Referent	
387.87 – 494.98 µg	0.77 (0.40, 1.47)	0.51 (0.24, 1.08)	
494.98 – 627.35 µg	0.43 (0.22, 0.84)	0.28 (0.12, 0.64)	
> 627.35 µg	0.52 (0.26, 1.01)	0.26 (0.084, 0.78)	
Age			**0.09**
≤ 55	Referent	Referent	
56-65	1.66 (0.61, 4.53)	2.11 (0.7, 6.33)	
66-75	1.55 (0.56, 4.29)	1.94 (0.64, 5.94)	
> 75	3.61 (1.01, 12.88)	5.6 (1.38, 22.67)	
Race			**0.19**
White	Referent	Referent	
Black	3.08 (0.91, 10.49)	3.39 (0.9, 12.79)	
Other	0.64 (0.22, 1.88)	0.93 (0.29, 3.01)	
BMI			**0.07**
< 25	Referent	Referent	
25-29	0.89 (0.45, 1.76)	0.71 (0.35, 1.47)	
30-34	0.89 (0.44, 1.77)	0.79 (0.38, 1.64)	
≥35	0.36 (0.15, 0.82)	0.31 (0.13, 0.78)	
Family history of PCa	2.44 (1.06, 5.64)	2.55 (1.04, 6.24)	**0.04**
Vitamin B6 intake (mg)	0.85 (0.63, 1.13)	1.33 (0.83, 2.13)	**0.24**

Prostate Cancer Cases Compared to Biopsy Negative Controls

In the comparison of cases with biopsy negative subjects, age, race, family history of prostate cancer, PSA, smoking, and education were found to be significantly associated with prostate cancer at the univariate level. All of these variables were included in the initial multivariate models along with blood folic acid concentration. Although no measure of dietary folate intake was significant at the univariate level,

because of the focus of this study on folate an initial multivariate model was built to contain dietary folate equivalents (Table 9).

Table 9. Initial multivariate model comparing prostate cancer cases with biopsy negative controls

Variable	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Blood folic acid concentration			**0.67**
≤ 504 ng/mL	Referent	Referent	
504 – 783 ng/mL	0.78 (0.42, 1.45)	0.91 (0.45, 1.83)	
783 – 1098 ng/mL	0.73 (0.39, 1.38)	0.85 (0.41, 1.73)	
> 1098 ng/mL	1.15 (0.62, 2.12)	1.27 (0.62, 2.59)	
Age			**0.005**
≤ 55	Referent	Referent	
56-65	2.02 (0.79, 5.19)	2.72 (0.94, 7.86)	
66-75	2.27 (0.87, 5.9)	2.9 (0.97, 8.64)	
> 75	14.08 (3.37, 58.83)	17.51 (3.58, 85.65)	
Race			**0.07**
White	Referent	Referent	
Black	2.58 (0.87, 7.63)	3.44 (1.0, 11.89)	
Other	0.42, 0.16, 1.14)	0.55 (0.19, 1.61)	
Family History of PCa	0.95 (0.49, 1.83)	1.08 (0.53, 2.22)	**0.83**
PSA			**0.55**
< 4	Referent	Referent	
4 - 10	1.2 (0.65, 2.2)	1.14 (0.59, 2.2)	
> 10	2.14 (0.99, 4.64)	1.58 (0.67, 3.73)	
Smoking status			**0.04**
Never	Referent	Referent	
Former	2.16 (1.17, 3.98)	2.01 (1.04, 3.89)	
Current	2.64 (1.32, 5.3)	2.62 (1.21, 5.71)	
Education			**0.08**
≤ High School	Referent	Referent	
Some/Technical College	0.44 (0.26, 0.74)	0.59 (0.32, 1.11)	
College Graduate	0.45 (0.26, 0.8)	0.59 (0.3, 0.94)	
Vitamin B6 intake (mg)	0.096 (0.75, 1.25)	1.31 (0.83, 2.07)	**0.24**
Dietary folate equivalents			**0.47**
≤ 387.87 µg	Referent	Referent	
387.87 – 494.98 µg	1.06 (0.59, 1.91)	0.76 (0.38, 1.54)	
494.98 – 627.35 µg	0.84 (0.45, 1.57)	0.59 (0.26, 1.32)	
> 627.35 µg	0.72 (0.39, 1.33)	0.43 (0.14, 1.27)	

In the initial multivariate model, dietary folate equivalents was not significantly associated with prostate cancer and was excluded from the final model. Vitamin B6 intake was retained to provide adjustment relative to blood folic acid concentration, although neither of these variables was significant in the multivariate models. All other variables remained significant except family history and PSA; PSA was eliminated whereas family history was retained due to its clinical significance. The final model included blood folic acid concentration, age, race, smoking, education, and vitamin B6 (Table 10).

Subjects in the three highest age categories were more likely to have prostate cancer than those under 55 years of age, although the association was significant only for the comparison of those greater than 75 years compared to those under 55 years (OR= 17.26, 95% CI 3.66 – 81.33). Black subjects were more likely to have prostate cancer than white subjects with an odds ratio of 3.66 (95% CI 1.09 – 12.25). Both former and current smokers were more likely to have prostate cancer than never smokers with odds ratios of 1.96 and 2.82, respectively (95% CI 1.02 – 3.76 and 1.31 – 6.05, respectively). Finally, subjects who attended some college or technical college and those who were college graduates were less likely to have prostate cancer than those with a high school education or less, although the association was significant only for those with some college/technical college education (OR = 0.52, 95% CI 0.3 – 0.91). This model does not exhibit significant lack of fit according to the Hosmer-Lemeshow goodness of fit test ($X^2_8 = 2.94$, $p = 0.94$).

Table 10. Final multivariate model comparing prostate cancer cases with biopsy negative controls

Variable	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Blood folic acid concentration			**0.69**
≤ 504 ng/mL	Referent	Referent	
504 – 783 ng/mL	0.78 (0.42, 1.45)	0.9 (0.46, 1.8)	
783 – 1098 ng/mL	0.73 (0.39, 1.38)	0.8 (0.4, 1.62)	
> 1098 ng/mL	1.15 (0.62, 2.12)	1.2 (0.59, 2.41)	
Age			**0.004**
≤ 55	Referent	Referent	
56-65	2.02 (0.79, 5.19)	2.62 (0.92, 7.44)	
66-75	2.27 (0.87, 5.9)	2.84 (0.97, 8.31)	
> 75	14.08 (3.37, 58.83)	17.26 (3.66, 81.33)	
Race			**0.04**
White	Referent	Referent	
Black	2.58 (0.87, 7.63)	3.66 (1.09, 12.25)	
Other	0.42, 0.16, 1.14)	0.5 (0.18, 1.43)	
Family History of PCa	0.95 (0.49, 1.83)	1.11 (0.54, 2.26)	**0.78**
Smoking status			**0.03**
Never	Referent	Referent	
Former	2.16 (1.17, 3.98)	1.96 (1.02, 3.76)	
Current	2.64 (1.32, 5.3)	2.82 (1.31, 6.05)	
Education			**0.05**
≤ High School	Referent	Referent	
Some/Technical College	0.44 (0.26, 0.74)	0.52 (0.3, 0.91)	
College Graduate	0.45 (0.26, 0.8)	0.55 (0.3, 1.02)	
Vitamin B6 intake (mg)	0.096 (0.75, 1.25)	0.99 (0.74, 1.31)	**0.92**

Stratification by Gleason Score

Because risk factors for prostate cancer may vary according to severity of disease, I stratified the comparison of cases with clinic controls according to Gleason score (< 7, n=57, and ≥ 7, n=60). Gleason score was not available for three cases; these subjects were excluded from this analysis. A Gleason score of 7 or greater conveys an increased risk of metastasis and recurrence [28]. Stratification of the results of this study indicated that risk factors may indeed vary according to disease severity. Among men with Gleason ≥ 7, age, race, BMI, and family history of prostate cancer were significant predictors of prostate cancer (Table 11). In contrast, among men with Gleason < 7, only total drinks of alcohol was a significant predictor whereas the known risk factors of age, race, and family history were not (Table 12). In addition, although Dietary Folate Equivalents was significant in the univariate analysis, this association was no longer significant after adjustment for vitamin B6. Blood folic acid concentration was not associated with either category of prostate cancer severity but was retained in both models. As with the other multivariate models, vitamin B6 intake was included to provide adjustment relative to the blood folic acid measure.

Table 11. Multivariate model comparing severe prostate cancer cases (Gleason ≥ 7, n=60) with clinic controls (n=181)

Variable	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Blood folic acid		**0.8**		**0.41**
≤ 504 ng/mL	Referent		Referent	
504 – 783 ng/mL	1.3 (0.58, 2.91)		2.003 (0.8, 4.99)	
783 – 1098 ng/mL	0.85 (0.36, 2.02)		1.08 (0.42, 2.79)	
> 1098 ng/mL	1.11 (0.49, 2.51)		1.13 (0.46, 2.8)	
Age		**0.07**		**0.056**
≤ 55	Referent		Referent	
56-65	2.35 (0.5, 10.96)		2.88 (0.49, 16.79)	
66-75	2.33 (0.5, 10.96)		2.95 (0.5, 17.31)	
> 75	7.5 (1.32, 42.76)		10.3 (1.43, 74.14)	
Race		**0.023**		**0.02**
White	Referent		Referent	
Black	1.21 (0.33, 4.47)		7.1 (1.78, 28.3)	
Other	6.99 (1.2, 40.82)		0.9 (0.21, 3.78)	
BMI		**0.035**		**0.031**
< 25	Referent		Referent	
25-29	0.63 (0.28, 1.45)		0.49 (0.2, 1.19)	
30-34	0.84 (0.37, 1.88)		0.69 (0.29, 1.67)	
≥35	0.18 (0.05, 0.6)		0.15 (0.04, 0.56)	
Family history of PCa	2.63 (0.99, 7.01)	**0.053**	3.59 (1.19, 10.78)	**0.023**
Vitamin B6 intake (mg)	0.95 (0.66, 1.38)	**0.8**	1.04 (0.68, 1.58)	**0.87**

Table 12. Multivariate model comparing less severe prostate cancer cases (Gleason < 7, n=57) with clinic controls (n=181)

Variable	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Blood folic acid		**0.89**		**0.89**
≤ 504 ng/mL	Referent		Referent	
504 – 783 ng/mL	0.84 (0.35, 2.02)		1.06 (0.42, 2.65)	
783 – 1098 ng/mL	1.0 (0.43, 2.29)		1.13 (0.45, 2.83)	
> 1098 ng/mL	1.18 (0.53, 2.64)		1.38 (0.59, 3.21)	
Dietary folate equivalents		**0.06**		—
≤ 387.87 µg	Referent		—	
387.87 – 494.98 µg	0.57 (0.25, 1.29)		—	
494.98 – 627.35 µg	0.31 (0.13, 0.75)		—	
> 627.35 µg	0.48 (0.21, 1.1)		—	
Age		**0.88**		**0.83**
≤ 55	Referent		Referent	
56-65	1.27 (0.39, 4.13)		1.31 (0.36, 4.74)	
66-75	1.06 (0.32, 3.55)		1.03 (0.27, 3.86)	
> 75	1.67 (0.33, 8.37)		1.68 (0.3, 9.34)	
Race		**0.67**		**0.93**
White	Referent		Referent	
Black	1.96 (0.43, 9.05)		0.79 (0.08,. 8.22)	
Other	1.5 (0.11, 21.31)		0.77 (0.16, 3.8)	
Smoking Status		**0.15**		—
Never	Referent		—	
Former	2.71 (0.99, 7.45)		—	
Current	2.13 (0.70, 6.48)		—	
Alcohol (drinks per day)	1.24 (1.04, 1.49)	**0.02**	1.25 (1.04, 1.51)	**0.019**
Family history of PCa	2.39 (0.87, 6.61)	**0.09**	2.14 (0.71, 6.48)	**0.18**
Vitamin B6 intake (mg)	0.78 (0.53, 1.13)	**0.19**	0.68 (0.45, 1.03)	**0.07**

DISCUSSION

Although these data showed no significant association between blood folic acid concentration and prostate cancer, the research question remains important and is worthy of continued future study. In addition, the results of this study lead to intriguing questions about associations between the variables examined.

Blood folic acid concentration

Blood folic acid concentration was not significantly associated with prostate cancer in either the comparison of cases with clinic controls or the comparison of cases with biopsy negative controls. There are several possible explanations for this observation. First, the null association could be true. Second, the association could exist, but have smaller magnitude than we had power to detect, given the sample size of this study. Third, the assay we used to measure blood folic acid concentration could have been inaccurate or inadequately sensitive. Finally, given recent discussions on the possibility of differential action of folate depending on the timing of exposure, it is possible that the association between blood folic acid concentration and prostate cancer may only be evident with measurements preceding carcinogenesis [29].

The third explanation could be plausible for a few reasons. Red blood cell folic acid concentration is an accurate measure of body stores [30], however we were able to measure whole blood folic acid only, as the hematocrit of the sample was not measured. In addition, we measured only one replicate of each sample, and given the variation seen in control samples (intraassay and interassay CVs were 6.6% and 12.1%, respectively) additional replicates would have improved the accuracy of the

measurements. Furthermore, it is unclear whether the folic acid binding protein (FBP) used in the assay has the same binding affinity for all forms of folate that may be present in the sample. It is known that some FBPs exhibit higher affinity for oxidized forms of folic acid than for reduced folate [31]. The Immulite assay used in this study includes a pre-treatment step with dithiothreitol (DTT), a reducing agent, to ensure similar binding affinities for folate and folic acid, however I was unable to verify binding affinities for this particular FBP.

It is not surprising that blood folic acid concentration was not highly correlated with any of the dietary intake measures when evaluated as continuous or categorical variables (quartiles). One other study of folate and prostate cancer reported low correlation between serum folate and reported intake among participants with a correlation coefficient of 0.20 [22]. One study of NHANES data also reported low correlation between reported dietary intake of folate and both serum and RBC concentrations; reported correlation coefficients ranged from 0.11 to 0.29 in different subpopulations [32]. This is likely due in part to the different ways questionnaires and biochemical assays measure folate. Questionnaires may ask for reported intake during the previous day, week, or year, and may be given at a different time point than the blood or serum sample is taken. Consistency in the measurement of both dietary and biochemical indicators of folate status would improve these disparities.

Recent studies have suggested that natural and supplemental folic acid may act differently with regard to prostate cancer risk. In two randomized controlled trials, supplemental folic acid was shown to increase risk of both colorectal and prostate

cancer whereas baseline dietary intake of folate was associated with a decreased risk of prostate cancer in the Aspirin/Folate Polyp Study[8,23]. In addition, recent analysis of the United States and Canada, both of which have folic acid fortification programs, has found an increase in colorectal cancer prevalence since the fortification programs began [33]. Although the data in this study do not support a positive association between folic acid and prostate cancer, those studies that do report such an association should not be ignored. It is clear that further studies are necessary to elucidate the effects of both dietary folate and folic acid on cancer risk.

Comparison of Cases and Clinic Controls

In the multivariate model comparing prostate cancer cases with clinic controls, Dietary Folate Equivalents (DFE), BMI, and family history of prostate cancer were significantly associated with prostate cancer, whereas blood folic acid concentration, age and race were not. Subjects with both the highest and second-highest quartile of DFE were less likely to have prostate cancer than those with the lowest quartile of DFE. It is interesting that DFE was significantly associated with prostate cancer whereas the components of DFE, food folate and synthetic folate, were not significant. This suggests that although both food and synthetic folate may contribute to prostate cancer risk, the combination of these two sources of folate, taking into account their different bioavailability, is a stronger contributor than either of the individual components.

Increasing BMI was associated with a reduced odds of having prostate cancer. This association can be attributed to the difference in proportions of subjects in the highest category of BMI (>35) in cases and clinic controls. 23.8% of clinic controls had

BMI >35 whereas only 10.8% of cases were in this BMI category. Proportions of subjects in other BMI categories were more similar in cases and clinic controls. Interestingly, although some studies have reported a null association between BMI and prostate cancer, other studies have reported that obesity may be associated with aggressive prostate cancer [34].

Family history of prostate cancer is known to be a strong predictor of prostate cancer, and our results are consistent with this. Those with a family history of PCa were 2.55 times more likely to have prostate cancer than those without a family history. As studies of genetic factors of prostate cancer continue to be published, it will be most interesting to learn which factors exhibit strong associations. As of yet, reported family history of prostate cancer is a stronger predictor of prostate cancer than any single-nucleotide polymorphism currently known to be associated with prostate cancer [35].

Stratification by Severity of Disease

The Gleason scoring system provides a useful way to examine high-grade and low-grade prostate cancer. This system divides prostate cancer into five histologic patterns, ranging from well-differentiated prostate adenocarcinoma (grade 1) to poorly differentiatied lesions (grade 5). The score, which can range from 2-10, is the sum of the two most commonly seen grades. Gleason score is strongly associated with prostate cancer mortality; in a study of 767 men in Connecticut, the mortality rate for those with Gleason score of 2-4 was 60 times lower than the mortality rate for those with Gleason score of 8-10 [36]. Those with Gleason score of 5 or 6 had an intermediate risk of prostate cancer death.

Stratification of the comparison of cases and clinic controls yielded intriguing results. In the overall comparison of cases and clinic controls, Dietary Folate Equivalents, BMI, and family history were significantly associated with prostate cancer. After stratification, the established risk factors of age, race, and family history were significant only when examining severe disease (Gleason ≥ 7). BMI was also significantly associated with an increased risk of severe disease; in this population a BMI of 35 or greater was associated with an 85% decreased likelihood of having prostate cancer (95% CI 44% – 96%). However, the significance of this association is questionable due to the low number of severe cases with BMI ≥ 35 (n=4).

In contrast, the only variable that was significantly associated with less severe disease (Gleason < 7) was alcohol consumption. In this population, increasing alcohol consumption was associated with an increased likelihood of having prostate cancer; each drink consumed increased the odds by 0.25 (95% CI 0.04 – 0.51 increase). Alcohol consumption is known to increase the risk of certain types of cancer, but the effect of alcohol on prostate cancer risk is unclear [37,38]. In this study population alcohol consumption was associated with an increased risk of less severe prostate cancer, but no association was evident with more severe prostate cancer. Due to the high number of subjects who did not consume alcohol at all, the sample size was limited for this analysis. Due to the inconsistency of published data on the association between alcohol consumption and prostate cancer, further studies are recommended.

It was also interesting that although Dietary Folate Equivalents was significantly associated with less severe PCa in univariate analysis, this association became

nonsignificant after adjustment for Vitamin B6 intake. This has been seen in other studies examining folate and prostate cancer, and due to the relationship of these two B vitamins in one-carbon metabolic pathways, this is perhaps not surprising.

Comparison of Cases and Biopsy Negative Controls

In the multivariate model comparing prostate cancer cases with biopsy negative controls, age, race, smoking status, and education were significantly associated with prostate cancer, whereas blood folic acid concentration and family history were not. No measure of dietary folate intake was significantly associated with prostate cancer. It is not surprising that family history was not significantly different between these two groups given that family history is one factor in making the decision to undergo a prostate biopsy.

Although PSA was significant in univariate analyses, the lack of significance of PSA in the multivariate model was not unexpected because all subjects in this comparison had an elevated PSA. PSA screening has become very common in the United States due to its strong association with prostate cancer and because it often detects cancer at an earlier stage than does digital rectal examination, and PSA >4 is commonly used as a determinant for referral to a urologist [39]. The incidence of prostate cancer increased sharply in the United States after PSA screening became widely adopted in the late 1980s and early 1990s [40]. PSA testing is not without limitations, however; as seen in these data, elevated PSA by itself does not exhibit high specificity as a predictor for prostate cancer, and current PSA testing is not able to distinguish between more and less aggressive prostate cancer [41,42]. Current research is examining whether different

isoforms of PSA may be associated with different grades of prostate cancer, and whether additional biomarkers may be combined with PSA to provide additional specificity.

Age is a known risk factor for prostate cancer, but the association in this population is not as straightforward as it may first appear. The proportion of cases and biopsy negative controls in the second and third age categories (56-65 and 66-75) is similar, but 10.8% of cases were >75 years of age whereas only 1.7% of biopsy negative controls were in this age group. It is common to perform a prostate biopsy only if a man's life expectancy is at least 10 years, and clinicians generally often recommend a biopsy for a person over 75 years only if they are at very high risk for prostate cancer and have other clinical evidence suggestive of a positive diagnosis [43]. Although aging is known to be an independent risk factor for prostate cancer and this likely contributed to the significance of this association, in this population the significance of age is also due in part to a "screening effect."

Race is also a known risk factor for prostate cancer. African-American men have higher rates of prostate cancer diagnosis and mortality compared to Caucasian men [1]. In this study African-American men were 3.66 more likely than Caucasian men to have prostate cancer (95% CI 1.1, 12.25 times). This is comparable to rates seen in the literature; for example in 2008 the incidence of prostate cancer among African American men was 1.58 times higher than that of Caucasian American men [1].

Education is not itself known to be a risk factor for prostate cancer, however education is often used as a marker of socioeconomic status (SES). SES has been found

to be associated with prostate cancer, however researchers suggest this is at least partially due to differential access to health care (and thus differential rates of screening) between those with high and low SES [44]. In this study population, disparities in access due to SES should be attenuated due to the standards of care in the Veterans Affairs system. Educational attainment and SES should continue to be studied to determine whether factors additional to health care access can account for the observed association.

Strengths and Limitations

The design of this study provided several important strengths. First, subjects completed all questionnaires and provided blood samples prior to scheduled biopsy to avoid potential bias stemming from a cancer diagnosis. Second, we examined three groups of subjects: men with positive biopsy results, men with negative biopsy results, and men with no history of prostate abnormalities. Third, because all subjects were patients at the PVAMC, we know that treatment was consistent and the likelihood of differential misclassification bias is low.

The major limitations of this study relate to the method of blood folic acid analysis. First, due to financial constraints we ran only one replicate of each sample. Although the IMMULITE system has been verified as accurate, multiple replicates always improve accuracy of results. Second, although red blood cell folate is an accurate indicator of body stores [30], we do not have hematocrit levels of participant samples and therefore can report only whole blood folic acid concentrations, which may not be a representative indicator of average blood folic acid levels.

Future Studies

Although examining the association between folate status and prostate cancer can be challenging, adding an assessment of gene methylation may prove enlightening. It has been shown that changes in dietary folate intake can affect methylation status in vitro and in vivo [45,46]. In the Netherlands Cohort Study on Diet and Cancer, colorectal cancer patients with low folate/high alcohol intake had higher prevalence of methylation of certain cancer-related genes than patients with high folate/low alcohol intake [13]. To date, no study has examined the association between folate and methylation status in prostate cancer patients.

To improve on the limitations of the blood folic acid assay, some studies have used high-performance liquid chromatography (HPLC), which is not only a more sensitive assay, but can also distinguish between folate and free folic acid [47]. Although costly, the sensitivity of this method provides significant advantage. In addition, due to potential seasonal variation in folate intake it would be interesting to assess folic acid concentration at multiple timepoints to compute a more accurate level of folate content throughout a year's time.

Conclusion

In this study, blood folic acid concentration was not associated with prostate cancer. This lack of association was constant when comparing prostate cancer cases with biopsy negative controls and with PSA-normal clinic controls, as well as in analyses stratified by severity of disease. Although some researchers have expressed concerns that folic acid fortification may increase the incidence of certain cancers, this study

provides no evidence that high folate status is associated with an increased risk of prostate cancer. On the contrary, when comparing prostate cancer cases and clinic controls, high intake of Dietary Folate Equivalents was associated with a decreased risk of disease. This association was not seen in stratified models. Further studies should be conducted to assess the impact of natural folate compared to synthetic and supplementary folic acid, as well as the impact of folate status at different time points.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. *CA Cancer J Clin*. 2008;58(2):71-96.

2. Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part II: individual countries. *BJU Int*. 2002;90(2):174-184.

3. Cook LS, Goldoft M, Schwartz SM, Weiss NS. Incidence of adenocarcinoma of the prostate in Asian immigrants to the United States and their descendants. *J Urol*. 1999;161(1):152-155.

4. U.S. Food and Drug Administration. Food standards: amendment of standards of identity for enriched grain products to require addition of folic acid (21 CFR Parts 136, 137, and 139). *Fed Regist*. 1996;61:8781-8801.

5. Dietrich M, Brown CJ, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake, and dietary folate sources among adult non-supplement users in the United States. *J Am Coll Nutr*. 2005;24(4):266-274.

6. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci U S A*. 1997;94(7):3290-3295.

7. Giovannucci E. Modifiable risk factors for colon cancer. *Gastroenterol Clin North Am*. 2002;31(4):925-943.

8. Cole BF, Baron JA, Sandler RS, et al. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *JAMA*. 2007;297(21):2351-2359.

9. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med*. 2003;349(21):2042-2054.

10. Herman JG, Graff JR, Myohanen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci U S A*. 1996;93(18):9821-9826.

11. Jeronimo C, Henrique R, Hoque MO, et al. A quantitative promoter methylation profile of prostate cancer. *Clin Cancer Res*. 2004;10(24):8472-8478.

12. Toyota M, Issa JP. The role of DNA hypermethylation in human neoplasia. *Electrophoresis*. 2000;21(2):329-333.

13. van Engeland M, Weijenberg MP, Roemen GM, et al. Effects of dietary folate and alcohol intake on promoter methylation in sporadic colorectal cancer: the Netherlands cohort study on diet and cancer. *Cancer Res*. 2003;63(12):3133-3137.

14. Ellinger J, Bastian PJ, Jurgan T, et al. CpG island hypermethylation at multiple gene sites in diagnosis and prognosis of prostate cancer. *Urology*. 2008;71(1):161-167.

15. Henrique R, Ribeiro FR, Fonseca D, et al. High promoter methylation levels of APC predict poor prognosis in sextant biopsies from prostate cancer patients. *Clin Cancer Res*. 2007;13(20):6122-6129.

16. Hultdin J, Van Guelpen B, Bergh A, Hallmans G, Stattin P. Plasma folate, vitamin B12, and homocysteine and prostate cancer risk: a prospective study. *Int J Cancer*. 2005;113(5):819-824.

17. Vlajinac HD, Marinkovic JM, Ilic MD, Kocev NI. Diet and prostate cancer: a case-control study. *Eur J Cancer*. 1997;33(1):101-107.

18. Pelucchi C, Galeone C, Talamini R, et al. Dietary folate and risk of prostate cancer in Italy. *Cancer Epidemiol Biomarkers Prev*. 2005;14(4):944-948.

19. Rossi E, Hung J, Beilby JP, Knuiman MW, Divitini ML, Bartholomew H. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. *Ann Epidemiol*. 2006;16(3):206-212.

20. Stevens VL, Rodriguez C, Pavluck AL, McCullough ML, Thun MJ, Calle EE. Folate nutrition and prostate cancer incidence in a large cohort of US men. *Am J Epidemiol*. 2006;163(11):989-996.

21. Johansson M, Appleby PN, Allen NE, et al. Circulating concentrations of folate and vitamin B12 in relation to prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition study. *Cancer Epidemiol Biomarkers Prev*. 2008;17(2):279-285.

44. Liu L, Cozen W, Bernstein L, Ross RK, Deapen D. Changing relationship between socioeconomic status and prostate cancer incidence. *J Natl Cancer Inst*. 2001;93(9):705-709.

45. Al-Ghnaniem R, Peters J, Foresti R, Heaton N, Pufulete M. Methylation of estrogen receptor alpha and mutL homolog 1 in normal colonic mucosa: association with folate and vitamin B-12 status in subjects with and without colorectal neoplasia. *Am J Clin Nutr*. 2007;86(4):1064-1072.

46. Jacob RA, Gretz DM, Taylor PC, et al. Moderate folate depletion increases plasma homocysteine and decreases lymphocyte DNA methylation in postmenopausal women. *J Nutr*. 1998;128(7):1204-1212.

47. Kalmbach RD, Choumenkovitch SF, Troen AM, D'Agostino R, Jacques PF, Selhub J. Circulating folic acid in plasma: relation to folic acid fortification. *Am J Clin Nutr*. 2008;88(3):763-768.

22. Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, et al. Null association between prostate cancer and serum folate, vitamin B(6), vitamin B(12), and homocysteine. *Cancer Epidemiol Biomarkers Prev*. 2003;12(11 Pt 1):1271-1272.

23. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. *J Natl Cancer Inst*. 2009;101(6):432-435.

24. Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum and whole-blood folate. *Clin Chem*. 1996;42(10):1689-1694.

25. Shannon J, Tewoderos S, Garzotto M, et al. Statins and prostate cancer risk: a case-control study. *Am J Epidemiol*. 2005;162(4):318-325.

26. Diet History Questionnaire. . http://riskfactor.cancer.gov/DHQ/. Updated 2007. Accessed April 16, 2009.

27. Melse-Boonstra A, West CE, Katan MB, Kok FJ, Verhoef P. Bioavailability of heptaglutamyl relative to monoglutamyl folic acid in healthy adults. *Am J Clin Nutr*. 2004;79(3):424-429.

28. D'Amico AV, Whittington R, Malkowicz SB, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. *JAMA*. 1998;280(11):969-974.

29. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? *Am J Clin Nutr*. 2008;87(3):517-533.

30. Sauberlich HE. Folate status of US population groups. In: Bailey LB, ed. *Folate in Health and Disease.* New York, NY: Marcel Dekker; 1995:171-194.

31. Henderson GB. Folate-binding proteins. *Annu Rev Nutr*. 1990;10:319-335.

32. Ford ES, Bowman BA. Serum and red blood cell folate concentrations, race, and education: findings from the third National Health and Nutrition Examination Survey. *Am J Clin Nutr*. 1999;69(3):476-481.

33. Mason JB, Dickstein A, Jacques PF, et al. A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer Epidemiol Biomarkers Prev*. 2007;16(7):1325-1329.

34. Rodriguez C, Freedland SJ, Deka A, et al. Body mass index, weight change, and risk of prostate cancer in the Cancer Prevention Study II Nutrition Cohort. *Cancer Epidemiol Biomarkers Prev*. 2007;16(1):63-69.

35. Fitzgerald LM, Kwon EM, Koopmeiners JS, Salinas CA, Stanford JL, Ostrander EA. Analysis of Recently Identified Prostate Cancer Susceptibility Loci in a Population-based Study: Associations with Family History and Clinical Features. *Clin Cancer Res*. 2009;15(9):3231-3237.

36. Albertsen PC, Hanley JA, Fine J. 20-Year Outcomes Following Conservative Management of Clinically Localized Prostate Cancer. *JAMA*. 2005;293(17):2095-2101.

37. Rohrmann S, Linseisen J, Key TJ, et al. Alcohol consumption and the risk for prostate cancer in the European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev*. 2008;17(5):1282-1287.

38. Sesso HD, Paffenbarger RS,Jr, Lee IM. Alcohol consumption and risk of prostate cancer: The Harvard Alumni Health Study. *Int J Epidemiol*. 2001;30(4):749-755.

39. Pienta KJ. Critical appraisal of prostate-specific antigen in prostate cancer screening: 20 years later. *Urology*. 2009;73(5 Suppl):S11-20.

40. Thompson IM, Ankerst DP. Prostate-specific antigen in the early detection of prostate cancer. *CMAJ*. 2007;176(13):1853-1858.

41. Ashley T. Using predictive value, sensitivity and specificity to interpret laboratory tests: PSA for the diagnosis of prostate cancer. *J Insur Med*. 2005;37(4):261-263.

42. Carter HB, Ferrucci L, Kettermann A, et al. Detection of life-threatening prostate cancer with prostate-specific antigen velocity during a window of curability. *J Natl Cancer Inst*. 2006;98(21):1521-1527.

43. Coley CM, Barry MJ, Fleming C, Fahs MC, Mulley AG. Early detection of prostate cancer. Part II: Estimating the risks, benefits, and costs. American College of Physicians. *Ann Intern Med*. 1997;126(6):468-479.